REMARKS

Introductory Comments

Reconsideration of the above-identified application in view of the above amendments and arguments set forth is respectfully requested.

Claims 17, 18, 38-41 and 43 are pending and under consideration. Claim 38 has been amended for grammatical reasons by inserting the word "and" between steps b-iii) and b-iv). No new matter has been added as a result of these amendments.

Applicants acknowledge with thanks the Examiner's withdrawal of the rejection of the claims under 35 U.S.C. § 112, second paragraph and 35 U.S.C. § 101, and the rejection under 35 U.S.C. § 103 regarding the Lapidus reference.

Rejection of Claims 17 and 18 Under 35 U.S.C. § 102(e)

Claims 17 and 18 are rejected under 35 U.S.C. § 102(e), as being anticipated by Wittwer *et al.*, U.S. Patent No. 6,232,079 (herein "Wittwer").

Specifically, the Examiner asserts that Wittwer teaches a method for detecting a target nucleic acid sequence in a test sample as claimed (column 6, lines 1-15, column 44, lines 24-38, column 44, lines 50-67, column 45, lines 1-12, column 45, lines 13-53 and column 44, lines 24-38). The Examiner states that despite Applicants' arguments against the rejection, the rejection is maintained for these reasons: Wittwer teaches a discontinuous temperature four-step PCR process as claimed (column 44, lines 53-58 where Wittwer teaches maintaining the reaction at a temperature of 94 °C to dissociate or denature the double-stranded nucleic acid sequences; maintaining the reaction at a temperature of 50 °C sufficient to anneal the primers to the target nucleic acid; maintaining the reaction for a time at a temperature of 72 °C sufficient to extend the primers; and

raising the temperature of the reaction to a temperature of 94 °C to monitor the primer extension product).

Applicants respectfully traverse this rejection.

The Examiner alleges that Wittwer, at column 44, lines 53-58, discloses the four steps of b-i) to b-iv) as claimed. The four steps of b-i) to b-iv) of claim 17 are:

b) performing the following cycle

(i) maintaining the reaction mixture for a time and at temperature sufficient to dissociate double stranded nucleic acid sequences,

(ii) maintaining the reaction mixture for a time and at a temperature to allow the PCR primers and probe to hybridize to the nucleic acid and thereby form primer hybrids and probe hybrids,

(iii) maintaining the reaction mixture for a time and at a temperature sufficient to dissociate the probe hybrids, if the probe is not completely complementary to the nucleic acid, but not sufficient to dissociate the primer hybrids,

(iv) raising the temperature of the reaction mixture to a temperature sufficient to activate the polymerase (emphasis added).

These four discrete steps using four discrete conditions (temperature and/or time) are within one cycle. This is in contrast to the amplification conditions described by Wittwer which are reiterated as follows:

"Cycling conditions were 94 °C for 0 sec (slope=20), 50 °C for 10 sec (slope=20), and 72 °C for 0 sec (slope=1) for 50 cycles followed by cooling to 45 °C and continuous fluorescence monitoring at a slope of 0.2 °C/sec to 94 °C (emphasis added)."

The Examiner concludes from this passage that Wittwer discloses the claimed method that requires the four discrete steps using four discrete conditions as reiterated above.

The passage cited above clearly states that the temperature conditions of 94 °C, 50 °C and 72 °C are within one cycle of the Wittwer method. From the language emphasized above, the temperature conditions of 45 °C and 94 °C in the Wittwer method are outside the cycle. Moreover, the PCR cycle disclosed by Wittwer includes only three discrete conditions. The fourth step disclosed by Wittwer is separate from and subsequent to PCR cycling. In contrast, Applicants' method specifically requires four discrete conditions for four different reasons in one cycle: i) to dissociate double stranded nucleic acid sequence, ii) to allow the PCR primers and probe to hybridize to the nucleic acid and thereby form primer hybrids and probe hybrids, iii) to dissociate the probe hybrids, if the probe is not completely complementary to the nucleic acid, but not sufficient to dissociate the primer hybrids, and iv) to activate the polymerase. Wittwer clearly does not disclose these four steps.

The different temperature conditions disclosed in Wittwer appear to be for separate thermal cycles. See for example, column 41, lines 11-20. Here it is disclosed that the samples were cycled through three discrete steps similarly as described at column 44, lines 53-58. Again, separate conditions outside the cycles are described by Wittwer (while Applicants' steps b-i) to b-iv) are within one cycle).

Additionally, Applicants would like to, respectfully, point out the following with respect to the Wittwer disclosure to the Examiner. Wittwer's method does not work for relative quantification in the particular circumstance when probes for the two sequences of interest cross-hybridize to both target sequences. For example, in Figure 45, Wittwer discloses a good correlation between the percentage of fluorescence signal (the area under the dF/dt curve) and the amount of input DNA when the two input DNAs come from widely disparate genes (the CFTR and neu genes as disclosed in Wittwer's examples are members of entirely distinct gene families that share little or no sequence

homology). However, in Figure 46B, one can see by visual inspection that the percentages of fluorescence signal (the area under the dF/dt curve) for the two peaks in the heterozygous Factor V Leiden sample are not close to 50:50. This is because of cross-hybridization of the two probes. Because the melting kinetics are not first-order, which is the basis of Wittwer's invention, the relative amount of signal for the two probes depends on both the relative and absolute amounts of the two targets. Thus, Wittwer does not disclose how to deconvolute melting curve data for this purpose, or even whether it is possible. In contrast, the methods of the instant invention enables quantification by substantially eliminating the noise related to cross-hybridization prior to signal detection.

Finally, although Wittwer discloses that their methods can detect two forms of a single nucleotide polymorphism that could also be used to detect different forms of insertion/deletions, however, these forms of insertion/deletions are only those that are contained within the span of the probe oligonucleotide. Therefore, these forms of insertion/deletion do not include those of greater than 50 nucleotides as detected by Applicants' method and recited in the instant claims.

Additionally, Applicants would like to point out the following with respect to the instant invention. Applicants' four-temperature PCR cycle enhances the ability to quantify the relative amount of: 1) two polymorphic forms of a gene (such as *CYP2D6*1* and *CYP2D6*4*), or 2) two genes that have extremely high homology (such as *CYP2D6* and *CYP2D7P*). The three-temperature PCR cycles such as those disclosed by Wittwer are useful to identify the presence or absence of polymorphic forms of a gene or genes that have extremely high homology, but are not useful at the time of the invention, to determine ratios (e.g. 2:1 or 3:2) of polymorphic forms of a gene or genes that have extremely high homology. In contrast, Applicants' third temperature in the claimed method allows for preferential melting of a mismatched probe-target hybrid in order to accomplish this.

For these reasons, Applicants respectfully request withdrawal of the rejection of claims 17 and 18 under 35 U.S.C. § 102(e), as being anticipated by Wittwer *et al.*, U.S. Patent No. 6,232,079.

Rejection of Claims 17-18 and 38-41 Under 35 U.S.C. § 102(e)

Claims 17-18 and 38-41 are rejected under 35 U.S.C. § 102(e), as being anticipated by Lapidus *et al.*, U.S. Patent No. 6,143,529 (herein "Lapidus").

Specifically, the Examiner asserts that with respect to claims 17 and 18, Lapidus teaches a method for detecting a target nucleic acid sequence as claimed (column 10, lines 29-67, column 11 lines 37-40, column 11, lines 64-67, column 12, lines 38-43 and column 13, lines 8-52). The Examiner also states that Lapidus discloses the subject matter of claims 38-41 and refers Applicants to various passages of the patent. The Examiner states that despite Applicants' arguments against the rejection, the rejection is maintained for the following reasons: Lapidus teaches a four-step PCR process at column 11, lines 37-40 where four discontinuous temperatures of 94 °C, 60 °C, 72 °C and 72 °C are used. Lapidus teaches that the PCR reaction comprises a target DNA:probe complex (DNA captured with a probe) and PCR primers (column 10, lines 54-67). The Examiner further states that the claims use the "comprising" format wherein any additional steps or elements could be included and would meet the limitations of the claims.

Applicants respectfully traverse this rejection.

The method of the present invention as disclosed by Applicants is described *supra*.

Column 11, lines 37-40 of Lapidus, which the Examiner alleges as disclosing the claimed method, is recited as follows:

"Samples were heated to 94 °C for 5 minutes, and then 40 cycles were conducted between 94 °C, 60 °C, and 72 °C (1 minute each) followed by one cycle at 72 °C for 5 minutes (emphasis added)."

This passage shows the deficiencies of Lapidus, similar to the Wittwer disclosure mentioned above. The fourth cited temperature condition is outside the cycles and for different purposes than those claimed by Applicants. Lapidus does not clearly disclose the reasons for the different temperature conditions. Assuming *arguendo* that the different temperature conditions of Lapidus is for denaturing and annealing in a PCR reaction, Lapidus does not disclose the combination of steps b-i) to b-iv) as claimed, especially steps b-iii) and b-iv) in one cycle. The different objective of Lapidus's method as compared to that of Applicants' method would prevent Lapidus from using the combination of steps as claimed.

The shortcomings of Lapidus's disclosed methods are similar to Wittwer's, with respect to the instant invention. The three-temperature PCR cycles such as those disclosed by Lapidus are useful to identify the presence or absence of polymorphic forms of a gene or genes that have extremely high homology, but are not useful at the time of the invention, to determine ratios (e.g. 2:1 or 3:2) of polymorphic forms of a gene or genes that have extremely high homology. In contrast, Applicants' third temperature in the claimed method allows for preferential melting of a mismatched probe-target hybrid in order to accomplish this.

Therefore, Applicants respectfully request withdrawal of the rejection of claims 17-18 and 38-41 under 35 U.S.C. § 102(e), as being anticipated by Lapidus *et al.*, U.S. Patent No. 6,143,529.

Rejection of Claim 35 Under 35 U.S.C. § 103(a)

Claim 35 is rejected under 35 U.S.C. § 103(a), as being unpatentable over Lapidus *et al.*, U.S. Patent No. 6,143,529 (herein "Lapidus") in view of Johansson *al.*, Pharmacogenetics, Vol. 6, pp. 351-355 (herein "Johansson").

Specifically, the Examiner asserts that Lapidus discloses all of the elements of the claim except detecting gene deletions or insertions in a CYP2D6 locus. However, the Examiner asserts that Johansson teaches distinguishing deletion of an entire region in a target nucleic acid which contains CYP2D6*5 (page 351, column 1, paragraph 1 and page 353, column 2, paragraph 1) to alter drug therapy and evaluate the linkage between CYP2D6 genotype and disease (page 354, column 2, paragragh 1).

Applicants respectfully traverse this rejection.

The deficiencies of Lapidus are stated *supra*. Applicants' arguments are incorporated herein. Applicants submit that Johansson does not remedy the deficiencies of Lapidus. Also, Applicants would like to point out to the Examiner that Johannson's method is not homogenous or automatable, two fundamental disadvantages that are overcome by the instant invention.

Accordingly, Applicants respectfully request withdrawal of the rejection of claim 35 under 35 U.S.C. § 103(a), as being unpatentable over Lapidus *et al.*, U.S. Patent No. 6,143,529 in view of Johansson *al.*, Pharmacogenetics, Vol. 6, pp. 351-355.

CONCLUSION

Applicants respectfully submit that the claims comply with the requirements of 35 U.S.C. Sections 102 and 103. Accordingly, a Notice of Allowance is believed in order and is respectfully requested.

Should the Examiner have any questions concerning the above, she is respectfully requested to contact the undersigned at the telephone number listed below. If the Examiner notes any further matters which the Examiner believes may be expedited by a telephone interview, the Examiner is requested to contact the undersigned.

If any additional fees are incurred as a result of the filing of this paper, authorization is given to charge deposit account no. 23-0785.

Respectfully submitted,

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